High Efficiency Yeast Transformation :

Overview :

This protocol is generously provided by Derek McCusker, PhD Group Leader, CNRS IBGC - UMR 5095 (CNRS-UBS).
This protocol is based on use of Polyethylene Glycol and Lithium Acetate.
Polyethylene glycol is indispensable for successful transformation of intact cells and the attachment of DNA and possibly acts on the membrane to increase the transformation efficiency. Both lithium acetate and heat shock, which enhance the transformation efficiency of intact cells, probably help DNA to pass through the cell wall.

Procedure :

For approximately 5-10 transformations :

1. Grow 100 mL of cells overnight to an optical density of between 0.5 and 1

2. Prepare LiOAc MIX and PEG MIX ahead of time

3. Split culture into two 50 ml conical tubes, pellet the cells, and remove the supernatant

4. Resuspend pellets in a total of 15 mls LiOAc MIX

5. Combine into a 15 ml tube, pellet the cells, and remove the supernatant

6. Resuspend pellets in 15 mls LiOAc MIX , pellet the cells, and remove the supernatant

7. Resuspend the pellet in 0.5 - 1 mls LiOAc MIX

8. Set up transformations. To each transformation add:
    - 30 µL of 5 mg/mL of Salmon Sperm DNA
    - 1-5 µg of specific DNA (1 µL of miniprep DNA)
    - vortex
    - add 100 µL cell suspension
    - vortex
    - add 0.7 mL PEG MIX
    - vortex

9. Incubate 60 min at 30°C

10. Heat shock 20 min at 42°C

11. Pellet cells and resuspend in 150 µL YPD

12. Plate on selective media

LiOAC MIX:
- 100 mM LiOAc
- 10 mM Tris (7.5)
- 1 mM EDTA

PEG MIX:
- 40% PEG, mw 3350
- 100 mM LiOAC
- 10 mM Tris (pH : 7.5)
- 1 mM EDTA